Effect of Radiations on Bacteriophage C_{16}

IT has been shown previously[1] that the effect of X-rays on phages is in relation to particle size, as determined by ultra-filtration and ultra-centrifugation analysis: the larger the particle size, the greater is the sensitiveness of the phage to radiation.

We have undertaken a quantitative analysis of this phenomenon, and are dealing here with the effect of different radiations on phage C_{16} (F. M. Burnet)[2], which is active on dysentery bacillus Y_{6R}. Its diameter, as determined by Elford and Andrewes, is 50–75 mμ.

The following radiations have been used: (1) monochromatic X-rays of 17 kv. (K_α line of molybdenum); (2) non-monochromatic hard X-rays (D.C., 200 kv.); (3) total radiation of radon + active deposit, dissolved in the phage suspension. The effects on the phage were followed by the plaque count method, which

D (r units)	N/N_0	$\sigma = \frac{-\log_e N/N_0}{D}$	$N/N_0 = e^{-\bar{\sigma}D}$ (calc.)
X-rays 17kv.			
$5{\cdot}0\times10^3$	0·83	$3{\cdot}72\times10^{-5}$	0·89
10^4	0·80	$2{\cdot}23\times10^{-5}$	0·80
$3{\cdot}0\times10^4$ (2 exp.)	0·56	$1{\cdot}92\times10^{-5}$	0·52
$4{\cdot}0\times10^4$	0·40	$2{\cdot}29\times10^{-5}$	0·41
$6{\cdot}0\times10^4$	0·35	$1{\cdot}75\times10^{-5}$	0·26
$7{\cdot}5\times10^4$	0·24	$1{\cdot}90\times10^{-5}$	0·19
$9{\cdot}0\times10^4$	0·16	$2{\cdot}03\times10^{-5}$	0·14
		$\bar{\sigma} = 2{\cdot}22\times10^{-5}$	
X-rays 200 kv.			
10^4	0·79	$2{\cdot}35\times10^{-5}$	0·775
$2{\cdot}0\times10^4$	0·64	$2{\cdot}23\times10^{-5}$	0·60
$4{\cdot}0\times10^4$	0·42	$2{\cdot}71\times10^{-5}$	0·36
$6{\cdot}0\times10^4$	0·21	$2{\cdot}58\times10^{-5}$	0·22
$7{\cdot}5\times10^4$	0·15	$2{\cdot}53\times10^{-5}$	0·15
$9{\cdot}0\times10^4$	0·075	$2{\cdot}85\times10^{-5}$	0·10
		$\bar{\sigma} = 2{\cdot}54\times10^{-5}$	
Radon (α-particles + β-rays)			
$5{\cdot}2\times10^4$ (2 exp.)	0·86	$2{\cdot}88\times10^{-6}$	0·79
$7{\cdot}3\times10^4$ (2 „)	0·70	$4{\cdot}88\times10^{-6}$	0·71
$11{\cdot}1\times10^4$ (1 „)	0·51	$6{\cdot}05\times10^{-6}$	0·59
$12{\cdot}2\times10^4$ (2 „)	0·58	$4{\cdot}37\times10^{-6}$	0·56
$17{\cdot}7\times10^4$ (2 „)	0·45	$4{\cdot}51\times10^{-6}$	0·44
$23{\cdot}3\times10^4$ (3 „)	0·28	$5{\cdot}45\times10^{-6}$	0·33
$37{\cdot}6\times10^4$ (2 „)	0·14	$5{\cdot}20\times10^{-6}$	0·17
$52{\cdot}5\times10^4$ (3 „)	0·09	$4{\cdot}60\times10^{-6}$	0·08
		$\bar{\sigma} = 4{\cdot}69\times10^{-6}$	

practically corresponds to counting the number of active particles present in the samples[3].

The results, shown in the accompanying table, may be summarized as follows:

(1) For all the radiations used, the proportion N/N_0 of active particles diminishes exponentially as dose D increases:

$$N/N_0 = e^{-\sigma D}. \quad (1)$$

It will be seen from the table that the values N/N_0 experimentally found are in very good agreement with those calculated on the assumption of an exponential law.

(2) The effect of radiations on phage is a function of the dose $D = I.t$, and does not depend on its components I (intensity) and t (time of exposure).

(3) The doses of X-rays being expressed in r units ($1r = 2 \times 10^9$ ions/cm.^{3} in air), equal doses of different wave-length rays produce the same effect.

(4) With α-particles a dose[4] seven times larger than with X-rays must be used in order to produce the same effect.

These results can be explained in terms of the 'target hypothesis'[5], in the following way.

A phage particle is inactivated by a single 'hit', as shown by the exponential inactivation rate. The independence of the wave-length in the case of X-rays shows that this hit is an elementary ionization process (single ionization, or (Jordan[6]) small groups of 2·2 ions in the average).

The relationship (1) allows us to calculate the value of σ. If we express the doses in ions (or in ion groups) per volume unit, σ has the dimensions of a volume ('action volume'): all the ionizations produced in this theoretical volume—and those only—are effective. In the case of X-rays the action volume has a radius of 14 mμ (doses expressed in ions) or 18 mμ (doses in ion groups). With α-particles the radius is reduced to 7·5 mμ (doses in ions).

In comparing the dimensions of the phage particles, as determined by ultra-filtration and ultra-centrifugation, with the calculated action volume, it should be borne in mind that the latter are by themselves but expressions of probability: we may imagine, for example, an action volume σ', n times larger, in which an ionization process is followed by inactivation with a probability $p = 1/n$; $(\sigma = p\sigma')$.

However, the fact that the calculated action volume is smaller in the case of radiations which produce a very dense ionization (α-particles), shows that the action volume corresponds to a really existing 'sensitive volume'[6]. When the distance between two ionizations is smaller than the dimensions of this sensitive volume, a part of the ionization process is ineffective for the biological action: correspondingly more energy must be absorbed, in order to produce the same effect. The sensitive volume will have, as lower limit, the dimensions of the action volume calculated for hard X-rays, and will be equal to it, if $p = 1$.

On the other hand, in the case of the very densely ionizing α-particles, it seems reasonable to think that the collision of such a particle with the sensitive volume is surely an effective 'hit'. Then, if we express doses in α-trajectories per surface unit, the value of σ from relationship (1) gives us the cross-section for this collision. The cross-section would have a radius of about 50 mμ. Deducting from that the radius of the ionization column produced by the α-particle (about 20 mμ; Jordan[7]), we find for the sensitive volume a radius of 30 mμ, in rather good agreement with the radius of the phage particles as given by ultra-filtration.

On the whole, our results tend to prove that phage inactivation is an elementary quantic process. They are in agreement with the view that phages, as well as certain viruses, are monomolecular structures: the inactivation should be conceived as a quantic transition of such a molecule. In this respect, our results are closely similar to those obtained by Timofeeff-Ressovsky[8] on gene radio-mutations in Drosophila.

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[1] Wollman and Lacassagne, *Ann. Inst. Past.*, **64**, 5 (1940).
[2] We owe this phage and the sensitive strain to the kindness of W. J. Elford.
[3] Luria, *Ann. Inst. Past.* (in the press).
[4] Calculated on the assumption that β-rays are as effective as X-rays.
[5] Holweck and Lacassagne, *C.R. Acad. Sci.*, **188** and **189** (1929–30); Lea, Haines and Coulson, *Proc. Roy. Soc.*, B, **120**, 47 (1935); Jordan, *Phys. Z.*, **39**, 345 (1938).
[6] *Arch. ges. Virusfors.*, **1**, 1 (1939).
[7] A recent estimation of Fano (unpublished, kindly communicated by the author) seems to show this value to be too high.
[8] Timofeeff-Ressovsky, Delbrück and Zimmer, *Göttinger Nach.*, Series 6, **1**, 190 (1935).